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REVIEW

Sex differences in autophagy-mediated diseases: toward precision medicine

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ABSTRACT

Nearly all diseases in humans, to a certain extent, exhibit sex differences, including differences in the onset, progression, prevention, therapy, and prognosis of diseases. Accumulating evidence shows that macroautophagy/autophagy, as a mechanism for development, differentiation, survival, and homeostasis, is involved in numerous aspects of sex differences in diseases such as cancer, neurodegeneration, and cardiovascular diseases. Advances in our knowledge regarding sex differences in autophagy-mediated diseases have enabled an understanding of their roles in human diseases, although the underlying molecular mechanisms of sex differences in autophagy remain largely unexplored. In this review, we discuss current advances in our insight into the biology of sex differences in autophagy and disease, information that will facilitate precision medicine.

Abbreviations: AD: Azheimer disease; AMBRA1: autophagy and beclin 1 regulator 1; APP: amyloid beta precursor protein; AR: androgen receptor; AMPK: AMP-activated protein kinase; ATG: autophagy related; ATP6AP2: ATPase H+ transporting accessory protein 2; BCL2L1: BCL2 like 1; BECN1: beclin 1; CTSD: cathepsin D; CYP19A1: cytochrome P450 family 19 subfamily A member 1; DSD: disorders of sex development; eALDI: enhancer alternate long-distance initiator; ESR1: estrogen receptor 1; ESR2: estrogen receptor 2; FYCO1: FYVE and coiled-coil domain autophagy adaptor 1; GABARAP: GABA type A receptor-associated protein; GLA: galactosidase alpha; GTEx: genotype-tissue expression; HDAC6: histone deacetylase 6; I-R: ischemia-reperfusion; LAMP2: lysosomal associated membrane protein 2; MAP1LC3B/LC3B: microtubule associated protein 1 light chain 3 beta; MTOR: mechanistic target of rapamycin kinase; m6A: N6-methyladenosine; MYBL2: MYB proto-oncogene like 2; PIK3C3: phosphatidylinositol 3-kinase catalytic subunit type 3; PSEN1: presenilin 1; PSEN2: presenilin 2; RAB9A, RAB9A: member RAS oncogene family; RAB9B, RAB9B: member RAS oncogene family; RAB40AL: RAB40A like; SF1: splicing factor 1; SOX9: SRY-box transcription factor 9; SRY: sex determining region Y; TFEB: transcription factor EB; ULK1: unc-51 like autophagy activating kinase 1; UVRAG: UV radiation resistance associated; VDAC2: voltage dependent anion channel 2; WDR45: WD repeat domain 45; XPDS: X-linked parkinsonism and spasticity; YTHDF2: YTH N6-methyladenosine RNA binding protein 2

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Introduction

To maintain cellular homeostasis, eukaryotic cells from yeasts to mammals have evolved a balance mechanism between the synthesis and degradation of various cellular substances. As a catabolic process, macroautophagy/autophagy is essential for maintaining cellular activities through the degradation and subsequent recycling of cellular proteins and organelles [1,2]. This process involves sequestration of cytoplasmic compartments by a double-membrane phagophore and delivery to the lysosome, where the captured cell parts are finally degraded by lysosomal proteases. Autophagy, on the one hand, can degrade harmful or superfluous cellular components, including both cytoplasmic and nuclear materials [3], while on the other hand, the degraded substances can be reused for cellular and tissue remodeling. Autophagy is a strictly regulated process that plays important roles under various physiological and pathological conditions. Accordingly, dysregulation of autophagy is implicated in various types of human disease [4], including cancer, neurodegenerative diseases, cardiovascular diseases, and immune and metabolic disorders. Recent studies have shown that sex differences exist in a broad range of disease entities, not only in the pathology of diseases, but also in pharmacokinetics and drug therapy. However, sex differences in autophagy and their roles in relevant diseases have been underestimated. Accumulating evidence suggests that autophagy regulates sexual differentiation, and that sex differences affect the roles of autophagy in health and disease. Thus, understanding the biology of sex differences in autophagy has important physiological and therapeutic implications.

Sex and gender aspects in precision medicine

In clinical practice, nearly all therapy has been designed based on a "one-size-fits-all" approach. This strategy is successful for some patients but not for others because it ignores

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individual differences, in particular those involving sex and gender. Precision medicine follows the principle of "right drug–right dose–right person" in medical treatments. Individual genome, sex/gender, environment, and lifestyle should be included in treatment programs. As a part of personalized medicine, gender medicine considers sex and gender differences in the onset, progression, prevention, and therapy of diseases, which matches the objectives of tailoring medicine to individual patients. Recently, a large-scale RNA-Seq analysis of somatic mutations in normal tissues from the GTEx project showed that breast tissue has a significantly higher number of somatic mutations in women than in men [5], implicating sex-biased somatic mutations in breast cancer. Because of significant differences between sexes in health and disease, NIH has developed a policy to require preclinical researchers to consider both females and males in cell and animal studies [6]. Gender medicine is a key part of precision medicine. With an increasing understanding of sex differences in medicine, people attach increasing importance to gender medicine.

Sex differences in diseases

Females and males have different genome constitutions and distinct modes of relevant gene expression regulation. Thus, considerable differences in genetics, anatomy, and physiology between females and males lead to sex differences in the development and prevention of diseases, the awareness and presentation of symptoms, and the effectiveness of therapy.

Sex differences exist in nearly every aspect of disease from mechanism, occurrence, and development to therapy and prognosis. For example, aspirin has different preventative effects on stroke in women and men, women suffering from stress experience depression more frequently than men, and men rarely develop osteoporosis [7]. Major types of human diseases, which show significant differences between sexes, include those involved in neural, immune, endocrine, cardiovascular, respiratory, hepatic, urinary, digestive, hematological, and reproductive systems [7]. For example, obesity, type II diabetes, autoimmune, cardiovascular diseases, Alzheimer disease (AD), arthrosis and cancer show sex differences. Diseases with sex differences are associated with major human organs, such as brain, heart, lung, liver, kidney, gut, pancreas, blood, and bone, in addition to sex-specific organs, such as the testis, prostate, ovary, and breast (Figure 1(A)).

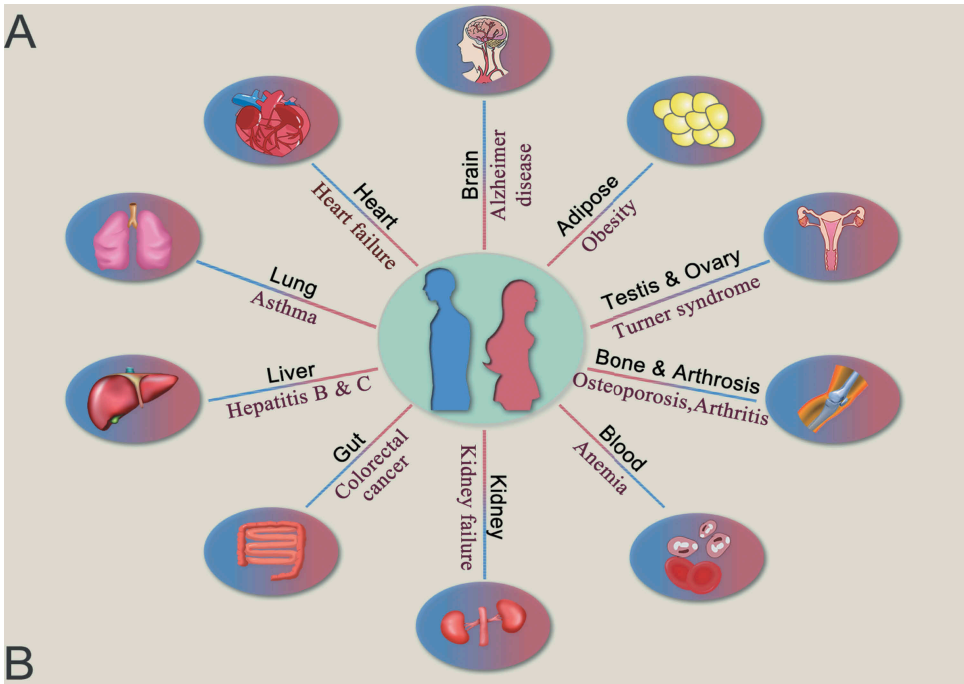
Cardiovascular diseases are the number one causes of death worldwide, accounting for 31% of all deaths (www.who.int), in which heart failure, myocardial infarction, and hypertension differ between females and males. The death percentage in cardiovascular diseases is higher for women than for men (51% vs 41%) [8]. Clinically, men experience severe coronary artery disease when they are young. However, women often develop cardiovascular diseases together with diabetes later in life [9]. We should consider sex as a risk factor for cardiovascular diseases, although the molecular mechanisms of sex effects on cardiovascular diseases need to be further explored. Interestingly, cardiovascular diseases are associated with autophagy regulation. For example, autophagy is impaired in ischemia-reperfusion (I-R) injury in the heart, which can be restored by enhancing autophagy via upregulation of the autophagy protein BECN1 [10]. In addition, androgens can regulate autophagy during myocardial infarction, based on the fact that age-specific incidence of ischemic heart disease in men is higher than in women [11]. These data suggest that autophagy is involved in sex-related cardiovascular diseases.

Cancer is the second most common cause of death worldwide. Cancer incidence and mortality rates are generally higher in men than in women. A total of 1,762,450 new cancer cases and 606,880 cancer deaths were predicted to occur in the United States in 2019 [12]. These cancers show sex differences in prevalence and categories (Figure 1(B)). Breast cancer in females and prostate cancer in males are associated with sex hormones and their receptors [13–15]. A recent study showed that tumor-suppressor genes that escape from X-inactivation are associated with cancer sex bias [16]. Hepatocellular carcinoma shows a male:female ratio averaging between 2:1 and 4:1 [17]. Thus, many cancers seem to be sex-biased diseases, although little is known about this concept. In addition, cancers that display sex-related biases are associated with autophagy. For example, in breast and ovarian cancers, frequent loss-of-heterozygosity of *BECN1* has been observed, and 40–75% of these two types of cancers have mono-allelic deletions in this gene [18].

Alzheimer disease presents frequently in women and is associated with depression and cognitive deterioration [19,20]. Evidence shows that decreased estrogen levels are relevant to the increased risk of AD [19]. For example, an increased risk of this disease is associated with an age-related loss of sex hormones [21]. In addition, sex chromosome complement has an effect on neurodegeneration in neurological diseases [22]. Accumulating evidence shows a role for autophagy deficiency in AD [23]. Thus, manipulating autophagy will probably be a new therapeutic tool in the treatment of this disease. Modulation of the autophagic pathway can be implemented by manipulation of sex steroids and their receptors AR (androgen receptor) and ESR (estrogen receptor), together with autophagy regulators such as MTOR [24].

Endocrine-related diseases are largely related to sex. Osteoporosis is a common disease in women after menopause, and fractures are a complication of osteoporosis, often leading to disability. Osteoporosis is an endocrine- and bone-related disease. Estrogen and ESR are associated with the onset of this disease, and men may also present with osteoporosis [7]. In the clinic, estrogen and bisphosphonates are often used in the treatment of osteoporosis [25]. In addition, autophagy is decreased in the endometrium of women with polycystic ovary syndrome, in which the autophagy proteins ATG3 and ATG14 are downregulated [26]. Autoimmune diseases, such as systemic lupus erythematosus, are more prevalent in women than in men. Serum estrogen levels are also associated with the development of this disease [27]. Steroid hormones are implicated in various types of human disease with sex differences; however, our understanding of diseases with sex differences is still limited.

Diabetes is a well-known risk factor for cardiovascular diseases. The risk factors for diabetes and cardiovascular complications include increased uric acid levels, low physical



B

Male	N	%
Prostate	174,650	20%
Lung & bronchus	116,440	13%
Colon & rectum	78,500	9%
Urinary bladder	61,700	7%
Melanoma of the skin	57,220	7%
Kidney & renal pelvis	44,120	5%
Non-Hodgkin lymphoma	41,090	5%
Oral cavity & pharynx	38,140	4%
Leukemia	35,920	4%
Pancreas	29,940	3%
All sites	870,970	

Female	N	%
Breast	268,600	30%
Lung & bronchus	111,710	13%
Colon & rectum	67,100	7%
Uterine corpus	61,880	7%
Melanoma of the skin	39,260	5%
Thyroid	37,810	4%
Non-Hodgkin lymphoma	33,110	4%
Kidney & renal pelvis	29,700	3%
Pancreas	26,830	3%
Leukemia	25,860	3%
All sites	891,480	

Figure 1. Major types of human diseases with sex differences. (A) Graphical depiction shows typical diseases with sex differences in various systems. Representative organs/tissues, such as brain, heart, lung, liver, gut, kidney, blood, bone, adipose, testis, and ovary, are associated with relevant diseases in men and women, such as obesity, cardiovascular diseases, and Alzheimer disease. (B) Schematic diagram of the most common cancers estimated in men and women in the United States in 2019. Case number and death percentage by sex are shown. Data are from the American Cancer Society 2019 [12].

activity and obesity, polycystic ovarian syndrome, and gestational diabetes in women [28]. However, these risk factors are quite different in men, and include smoking, alcohol abuse, systolic blood pressure, and hypogonadism [28]. Diabetes and related complications are often associated with obesity, and females and males store fat in different body regions. Men have more abdominal fat, whereas females have more fat in the hips and thighs. The fat distribution pattern of females is associated with lower cardiometabolic risk, and gluteofemoral fat loss and ectopic fat accumulation is evident in lipodystrophic syndromes [29]. There are also sex differences in fatty acid mobilization, oxidation and storage [30]. A recent study showed that the gut microbiome is associated with obesity and fat distribution [31]. Considering sex as a risk factor will benefit better treatment and prevention, although diabetes and obesity are quite complex diseases. Along these lines, it is important to consider sex difference in autophagy, which is implicated in the metabolic regulation of disease. For example, a sexual dimorphism in placental autophagy in response to maternal obesity is associated with offspring obesity [32]. Thus, sex differences in autophagy, at least in part, contribute to metabolic diseases, such as obesity and diabetes.

Sex determination and differentiation, the beginning of sex differences in diseases later in life

Sex influences all aspects of childhood, including differences in health, life span, cognitive abilities and responses to disease. How are sex determination and differentiation exactly related to sex differences? A fundamental factor for differences in physiology and disease between sexes is genetic constitution. Differences in females and males begin at the early stage of embryo development when sex is being determined. Females and males share 22 pairs of autosomes, but a pair of different sex

chromosomes, XX or XY. Thus, fundamental differences lay with the sex chromosomes. Human sex is determined by the female XX–male XY system with the key male-determining gene *SRY* on the Y chromosome [33]; males have a single X chromosome and a degenerate Y chromosome that bears *SRY*, and females have two X chromosomes.

When does the differentiation of the sex chromosomes X and Y begin in mammals? The origin of the human sex chromosomes has long attracted much interest due to their distinctive roles and features, involving not only sex determination and reproduction but also the development of higher cognitive abilities, immunity, and unique X dosage compensation [34]. A large number of X chromosome-associated diseases, such as hemophilia and color blindness, commonly emerge in humans due to the hemizygous heredity of males [35].

Following the trajectory of origin of the sex chromosomes can tell us their differences. Thus, a primary question concerns how and when the XX/XY difference originated. Actually, the split of the mammalian lineage from birds occurred over 310 million years ago. The sex chromosomes XX/XY in mammals and ZZ/ZW in birds evidently evolved from different pairs of autosomes independently within the last 350 million years [36–39]. During the evolutionary process, the X chromosome becomes relatively stable, but the Y chromosome is dynamic and degenerate. The Y chromosome is progressively degraded after acquiring a male-determining role, due to recombination suppression between chromosomes X and Y; thus, the modern Y chromosome in humans has fewer genes than its X counterpart (Figure 2(A)). The Y chromosome mainly retains the genes for enriching maleness [35,40,41]. Notably, several mammals have completely lost their Y chromosome [42,43]. The endpoint of Y degradation and the fate of the Y chromosome have sparked intense interest in recent years [40,44]. Thus, modern XY chromosomes in humans are still evolving, and variations and mutations often occur, particularly in the Y chromosome. For example, Y-chromosome microdeletions are frequently detected, which are one of major causes of male infertility [45].

In mammals, *SRY* on the Y chromosome is required for male physiological development [46]. When X-bearing sperm fertilize an X-bearing egg, the embryo has no *SRY* gene, and will default toward female development, whereas if Y-bearing sperm fertilize an X-bearing egg, the embryo will develop as a male because the *SRY* gene exists. (Figure 2(B)). SRY, as a transcription factor, induces testis differentiation and subsequent male development [46]. In the embryonic gonads of males, SRY and SF1 initiate SOX9 expression via the eALDI enhancer [47]. Once sexes are determined, all subsequent differences between sexes in humans are secondary effects owing to hormones/factors produced by the gonads, such as androgens and estrogens. These hormones act via their receptors, including AR, ESR1, and ESR2. Once an interaction with their target DNA sequences occurs in the nucleus, these receptors will activate transcription of the downstream genes through binding responsive elements in the respective

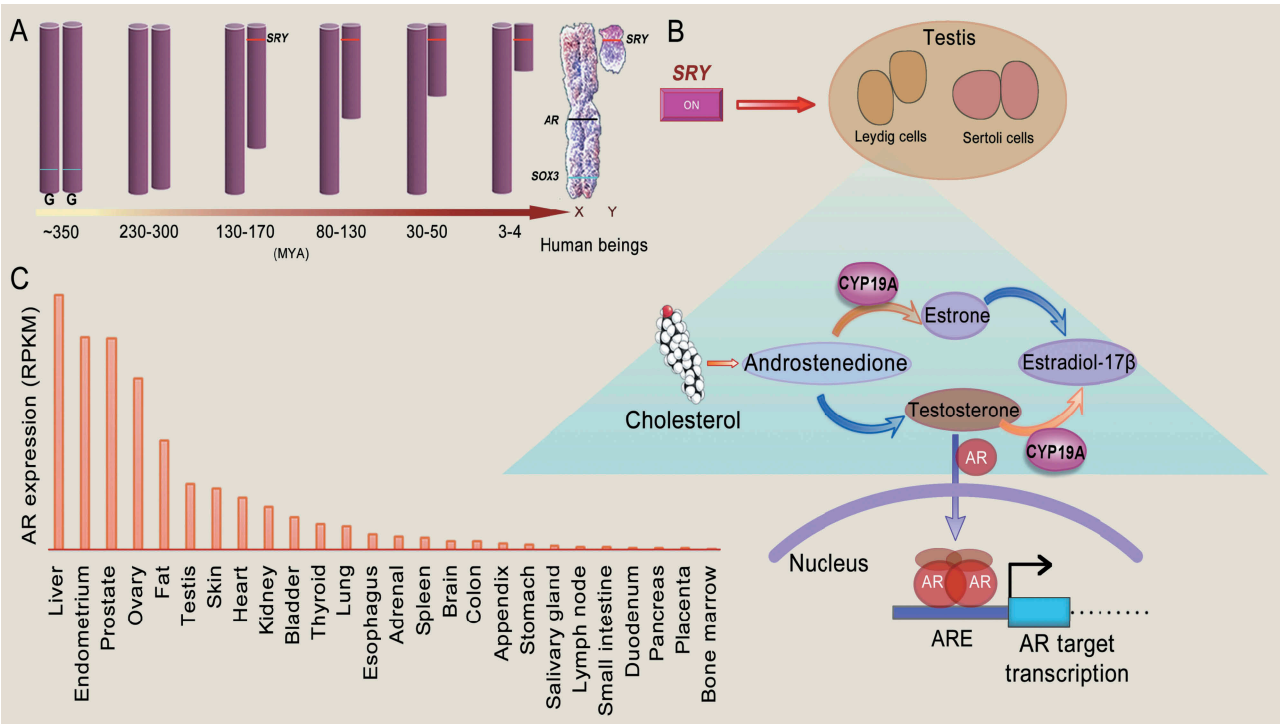


Figure 2. Sex determination and differentiation in human. (A) Schematic depiction of the evolution of sex chromosomes X and Y in humans, highlighting the differentiation of the X and Y chromosomes. XX determines female, whereas XY determines male. The X and Y chromosomes originated from an ordinary pair of autosomes (G/G) ~350 million years ago (MYA). During the evolutionary process, the X chromosome remains relatively stable, but the Y chromosome is dynamic and degenerate. The Y chromosome is progressively degraded after acquiring a male-determining role. Sex-determining gene *SRY* on the Y chromosome evolved from *SOX3* on chromosome X. (B) *SRY* starts male differentiation. If there is no *SRY*, the embryos will default toward female development. SRY, as a transcription factor, induces testis differentiation, and subsequent male development through supporting cells to produce sex hormones, such as androgens (testosterone) and estrogens (estradiol). These hormones act via binding their receptors, including AR, ESR1, and ESR2, in the nucleus. Then, they will activate transcription of their target genes to induce sex differentiation. (C) AR is expressed broadly in many tissues, not only in reproductive organs. RNA-Seq data were downloaded from BioProject: PRJEB4337.

promoters to induce sex differentiation (Figure 2(B) and (C)). Mutations in sex-determining genes and the dysregulation of relevant pathways will lead to disorders of sex development (DSD), including 46,XY complete or partial gonadal dysgenesis, 46,XY gonadal dysgenesis, 46,XX testicular DSD, and 46,XX ovotesticular DSD. Approximately 50% of these patients have errors in primary sex determination [33], highlighting the importance of sex differentiation in health and disease.

Sex differences in autophagy and relevant diseases

Autophagy is an intracellular degradation process that is utilized to recycle intracellular components through an autophago-some-lyso-some pathway when cells encounter stress. Autophagy shows sex differences in both physiological and pathological processes. For example, sex differences in autophagy have been observed in the Syrian hamster Harderian gland, which is regulated by redox-sensitive transcription factors [48]. In many biological processes, autophagy plays roles in a sex-biased manner. For instance, in surgically gonadectomized rats, the operation has no effect on ischemia-reperfusion-induced MTOR activation in male hearts, but promotes MTOR signaling in female hearts, suggesting that sex steroids regulate autophagy during myocardial infarction via MTOR signaling in female hearts [11]. Increasing evidence shows that sex differences in autophagy are implicated in many types of human diseases, such as cancer, cardiovascular diseases, and neurodegenerative diseases [49]. Some of these diseases will briefly be discussed in light of the roles of autophagy in diseases with sex differences (Table 1).

The most common cancer in females is breast cancer, which accounts for approximately one-third of all new cancer diagnoses in women [12]. Frequent loss-of-heterozygosity of *BECN1* has been observed in breast and ovarian cancers, and mono-allelic deletions occur in 40–75% of these two cancers [18]. BECN1 is a component of the class III phosphatidylinositol 3-kinase (PtdIns3 K) complex, which is thought to recruit effector proteins required for autophagosome formation, including BECN1, PIK3R4/VPS15, PIK3C3/VPS34, ATG14 and NRBF2 [50]. Considering the significant decrease in the protein levels of BECN1 in breast carcinoma cells compared with normal breast cells, decreased autophagy mediated by BECN1 could contribute to breast tumorigenesis. In addition, we established a functional connection among VDAC2, MYBL2, the BECN1-BCL2L1 pathway, and autophagy suppression in the developing ovary, which is required for follicular development [51]. BECN1 deficiency in the ovary can lead to a reduction in progesterone production and preterm labor [52]. These data suggest that autophagy is necessary for female fertility.

Alzheimer disease is the most common form of dementia, and the World Health Organization recommends AD research as a global public health priority. Several genes are involved in AD, including *APP, PSEN1* and *PSEN2* [53]. Sex differences in autophagy regulation contribute to a higher risk of AD and a greater severity of pathology in women [24]. There is increasing evidence that the dysregulation of autophagy is involved

Table 1. Roles of autophagy in diseases with sex differences.

Diseases	Roles of autophagy	Genes	Ref.
Cancers	A role for increased autophagy in prostate cancer progression.	*AR*	[78] [91] [92] [18,
	AR signalling promotes adaptation to nutrient starvation and, in turn, evasion of cell death.	*AR, HSPA5*	93]
	2-Methoxyestradiol induces autophagy in colon carcinoma.	*CYP19A1*	
	Autophagy acts as a tumor suppressor in hepatocellular, breast, ovarian, and prostate cancer.	*BECN1*	
Cardiovascular diseases	In cardiac atrophy, males lose more cardiac muscle than do females in cancer, and autophagy is the main proteolytic pathway involved.	*ESR, LC3B* *LC3B*	[94] [56] [10] [95] [96] [11 [97]
	In cardiac ischemia-reperfusion (I-R)-induced injury, autophagy is upregulated in females.	*BECN1*	
	Enhancing autophagy protects against I-R injury in the heart.	*AMPK,*	
	Ischemia stimulates autophagy via AMPK.	*BECN1*	
	Glycogen autophagy (glycophagy) is an important component of the response to cardiac metabolic stress in females.	*STBD1, AMPK* *AR, MTOR*	
	Sex steroids regulate autophagy during myocardial infarction.	*ATG5*	
	In coxsackievirus B3-induced myocarditis, sex-biased autophagy occurs in the heart, with females being substantially less susceptible than males.		
Chronic pancreatitis	Chronic pancreatitis is more common among men; impaired autophagy triggers chronic pancreatitis.	*ATG5*	[98]
Neural system diseases	Defective hypothalamic autophagy promotes obesity.	*ATG7, IKBKB*	[99] [100] [101] [24]
	FBXW7-AS1/DEspR haploinsufficiency with increased neuronal autophagy and cognitive decline in males.	*EDN1* *ESR1, ATG7*	[102] [103]
	ESR1 inhibits autophagy, leading to less severity of iron-induced brain injury in females than in males.	*MTOR, ESR1* *LAMP2,*	
	Lower levels of autophagy, increased risk of AD, and greater severity of pathology in women.	*TOMM20*	
	Decreased mitophagy in neonatal hypoxic-ischemic encephalopathy in males.	*ATG16L1*	
	SNPs of *ATG16L1* are associated with ankylosing spondylitis in females.		
Obesity	Activation of autophagy in placentas of males but not females from obese women.	*ATG7*	[32]
Osteoporosis	Autophagy deficiency aggravates the bone loss associated with ageing and decreased estrogen levels in women	*LC3B, ATG5*	[104]
Bowel diseases	In Crohn disease (CD), female-specific CD association in ATG16L1.	*ATG16L1*	[105]
Reproductive diseases	BECN1 deficiency in the ovary results in the reduction of progesterone production and preterm labor.	*BECN1* *ATG3, ATG14*	[52] [26] [106]
	Decreased autophagy in the endometrium of women with polycystic ovary syndrome.	*ATG5, ATG7,*	
	Disruption of autophagy by the Sertoli cell-specific knockout of *ATG5* or *ATG7* causes male subfertility.	*PDLIM1*	

in AD. Low levels of autophagy can lead to Aβ protein accumulation in neurons and astroglia cells, and pathological aggregates, in turn, further inhibit autophagy; thus, an increased Aβ protein accumulation could aggravate its harm to AD patients [24]. Interestingly, studies in primary human umbilical vein endothelial cells, by comparing the expression of BECN1 and the LC3-II:LC3-I ratio between sexes, show that male cells with one X chromosome have a greater autophagic capacity than female cells with two X chromosomes [54], although further experiments in vivo are needed to confirm this. Compared to males, lower levels of basal autophagy in females could indicate a higher risk of AD and could lead to a greater severity of pathology in women [24]. As AD is a protein-aggregation disease, the degradation of the aggregated proteins – or prevention of microaggregate formation – by autophagy represents a promising approach and therapeutic target in the treatment of AD.

Autophagy plays a vital role in the maintenance of heart function by removing dysfunctional cytosolic components and reusing degraded materials as a catabolic energy source. For example, neonatal starvation at birth can induce autophagy in the heart to ensure energy and nutrient supply [55]. In ischemia-reperfusion injury in the heart, both the formation and downstream lysosomal degradation of autophagosomes are impaired [10], and enhancing autophagy has a protective role against I-R injury. Moreover, autophagy shows sex differences in cardiac I-R-induced injury in rats: Female rats show a marked increase in LC3B, whereas males show significantly decreased autophagy in response to I-R [56]. In addition, age-specific incidence of ischemic heart disease in men is higher than in women, and androgens can regulate autophagy during myocardial infarction through the MTOR pathway [11]. These data suggest that autophagy is involved in sex-related cardiovascular diseases.

Roles of the X chromosome in the regulation of autophagy

X-linked diseases often occur more in men than in women. Actually, males are genetically hemizygotes because males have only one X chromosome, while females have two. Thus, phenotypes associated with mutations of the X-linked genes are often dominant in males, including in health and disease. Autophagy activities involved in the X-linked genes are, at least in part, sex-related, although causal relationships remain to be studied further. The functions of sex chromosomes are not only for sex determination and differentiation but also affect the regulation of autophagy. Several genes on the X chromosome participate in or affect autophagy activity, such as *ATP6AP2* (ATPase H+ transporting accessory protein 2) [57] and *LAMP2* (lysosomal associated membrane protein 2) [58], and mutations in these genes are involved in human diseases.

The RAB proteins belong to a small GTPase family that regulates vesicle trafficking. These small GTPases function through a switch between the GTP-bound (active) and the GDP-bound (inactive) forms, and many of these enzymes participate in autophagy [59,60]. There are at least seven X-linked *RAB* genes in humans, including *RAB9A, RAB9B, RAB33A, RAB39B, RAB40A, RAB40AL*, and *RAB41*. Of these, the RAB9A, RAB9B, RAB33A, and RAB39B gene products are involved in autophagy. RAB33A functions together with ATG16 L1 in autophagosome formation [61]. RAB39B is involved in autophagy initiation via ULK1 [62], and its mutations are responsible for X-linked mental retardation [63]. RAB9A and RAB9B play roles in mitophagy by alternative autophagy but not in conventional macroautophagy [64]. These data suggest that the X-linked RAB proteins are actively involved in various processes of autophagy regulation. The RABs act as modulators in the regulation of formation of autophagosomes and/or autolysosomes via vesicle trafficking. Given that RNAi-mediated knockdown of RAB33B has little effect on autophagosome formation [61], the RABs probably have alternative functions in the regulation of autophagy, as in the cases of RAB9A and RAB9B, or the regulatory processes are not rate-limiting in autophagy, and, instead, act only through facilitating autophagy via vesicle trafficking. In addition, RABs are factors with versatile functions in vesicular trafficking processes, and mutations of them mainly lead to neurological diseases [63]. Nevertheless, causal connections between these RAB-associated diseases and autophagy remain to be further studied.

ATP6AP2 is an X-linked novel gene associated with Parkinson disease, in particular X-linked parkinsonism with spasticity/XPDS [57]. This disease often occurs in male patients. ATP6AP2 is a vital accessory component of the vacuolar-type H^+-translocating ATPase (V-ATPase) required for autophagy. Loss-of-function mutations in ATP6AP2 impair V-ATPase function, leading to the accumulation of autophagosomes and defects in lysosomal clearance; thus, the onset of the disease presents as deposits of pathological proteins in neurons. Similar neurodegeneration and cognitive impairment are also observed in *atp6ap2/ATP6AP2* knockout models in mice and flies [65]. The KO male mice show autophagy defects that lead to axonal and neuronal degeneration, although female KO mouse models remain to be established to confirm sex differences in autophagy.

LAMP2 and GLA (galactosidase alpha) are lysosomal proteins. LAMP2 is a lysosomal protein involved in the fusion between autophagosomes and lysosomes. Loss-of-function mutations in LAMP2 cause Danon disease, and the patients suffer cardiomyopathy as a result of impaired autophagy [58]. The X-linked *GLA* gene is responsible for Fabry disease, a rare lysosomal storage disorder resulting from a deficiency of the corresponding lysosomal enzyme. An impaired autophagy flux is observed in fibroblasts derived from Fabry disease patients with *GLA* mutations [66].

WDR45/WIPI4 is an X-linked gene responsible for a neurodegenerative disorder, beta-propeller protein-associated neurodegeneration/BPAN [67]. WDR45 can interact with autophagic proteins ATG2A and ATG2B [68]. *wdr45* knockout mice show the accumulation of ubiquitin-associated aggregates in various brain regions [69], indicating impaired autophagy flux. *ATG4* is another X-linked gene, and several isoforms of the gene have roles in the lipidation of Atg8-family proteins (LC3 and GABARAP subfamilies) that is involved in autophagosome formation, but appear to have a redundant function [70].

HDAC6 is an X-linked gene that encodes a histone deacetylase. The main functions of this protein are histone deacetylation and transcription suppression. A recent study showed that

ciliophagy, an HDAC6-dependent autophagic pathway, is critical to cilia homeostasis [71]. In chronic obstructive pulmonary disease patients, excessive autophagy leads to a shortening of airway cilia upon oxidative stress. The HDAC6-mediated selective autophagy is a typical example of epigenetic regulation in autophagy. Recent data indicate that epigenetic regulations play important roles in establishing sex differences in both disease and health [7]. However, the underlying molecular mechanisms of these sex-differences in autophagy remain to be further explored. Future efforts may be to explore histone modifications, post-transcriptional modifications and genomic methylation under various conditions of physiology and pathology. In the future, researchers should also test and confirm that the expression changes of these genes on the X chromosome affect sex differences in autophagy activity and disease.

Regulation of autophagy by sex steroid hormone receptors

Autophagy shows sex-dependent differences in both physiological and pathological processes. In some disease types, autophagy plays a role in a sex-dependent manner. Increasing evidence indicates that the key mechanism in the sex-biased regulation of autophagy is through sex steroid hormones and their receptors. Given the vital contributions of AR, ESR1, and ESR2 to sex differences, we will briefly discuss their roles in the regulation of autophagy.

Androgens function via their receptor, AR, and estrogens act via their receptors, ESR1 and ESR2. AR, on chromosome X, is not only expressed in reproductive organs but also in many other tissues, mainly in liver, endometrium, prostate, ovary, fat and testis, based on transcriptome data in humans [72] (Figure 2(C)). In mice, AR mainly presents in bone marrow, brain, salivary gland, skin, spleen, thymus, heart, and adipose tissues, in addition to the testis, prostate, and epididymis in males, and the ovaries, uterus, and oviducts in females [73]. AR is also expressed in the eye, bladder, gut, stomach, muscle, lungs, and kidneys. This wide expression indicates that AR signaling is involved in a broad range of physiological processes.

ESR1 and ESR2 belong to a nuclear receptor superfamily of hormone receptors, and are expressed in different tissues and cell types. ESR1 is mainly expressed in the ovaries, uterus, pituitary gland, liver, hypothalamus, bone, mammary glands, cervix, lungs, and vagina [74]. ESR1 is also expressed in fat, thyroid, adrenal, prostate, and testis tissues. ESR2 is mainly expressed in ovary, testis, adrenal, lymph node, spleen, and fat tissues and is also present in the heart, brain, bone, lungs, prostate, gut, and bladder. These expression patterns show that ESR1 and ESR2 function in a distinct manner. For example, ESR1 and ESR2 are expressed in distinct brain regions in humans. ESR1, but not ESR2, plays an estradiol-induced protective role in the brain [75].

The importance of AR and ESR signaling outside the reproductive system is worthy of much attention. The dysregulation of AR and ESR signaling pathways is associated with a variety of human diseases that display sex differences, such as cancer, and neurodegenerative and cardiovascular diseases. Many of these diseases are relevant to dysfunctions in autophagy. Accumulating evidence shows that sex hormone receptors regulate many aspects of autophagy pathways. For example, AR signaling is closely associated with prostate cancer, in part through the dysregulation of autophagy [13]. An integrated and systems-level database for autophagy research that includes the transcriptional regulation of autophagy genes by AR and ESRs has been established [76]. Information resources in the autophagy studies [76,77] and JASPAR database V7 (aspar.genereg.net) are available for analysis of autophagy pathways through sex steroid hormones.

Accumulating evidence shows that AR and ESR1 are involved in the transcriptional regulation of several core autophagy genes that act in stages of phagophore induction, expansion, and maturation in humans (Figure 3). Components that participate in vesicle induction include ULK1, ULK2, and ATG13 that are within the ULK complex, and ATG14, PIK3C3 and AMBRA1 that are associated with the class III PtdIns3 K complex 1. Experiments confirm that AR can regulate the transcription of *ULK2, BCL2, AMBRA1*, and *PIK3C3* [78,79] (Figure 4). Interestingly, the transcriptional regulations mediated by AR are mainly achieved through binding to introns of target genes. For example, AR mediates the transcription of *ULK2* through binding to intron 23 of this gene, which is associated with prostate cancer [78]. Autophagy-related genes *BCL2, AMBRA1*, and *PIK3C3* were identified through ChIP-on-chip analysis of AR binding in primary human skeletal muscle myoblasts, suggesting roles of androgen signaling in muscle development, growth, and performance, in addition to potential therapeutic targets for treating muscle-wasting disorders [79]. In addition, experimental evidence shows that ESR1 can regulate the transcription of *BCL2* and *ULK1* [14,15] (Figure 4). Transcriptional activation of *BCL2* is mediated at the SP1 cis-element site via ESR1-SP1 interaction in the *BCL2* promoter, whereas ESR1 can bind to the *ULK1* promoter, hinting at a regulatory association of ESR1 with *ULK1* and *BCL2* in human breast cancer [14,15]. Thus, AR and ESR1 regulate phagophore induction through controlling many autophagy genes in the ULK complex and the PtdIns3 K complex 1 at the transcriptional level.

In phagophore expansion, AR mediates transcription of the core autophagy genes *ATG3, ATG4B, ATG5, ATG7*, and *LC3B*, as well as *SQSTM1* [79] (Figure 4). ChIP-on-chip analysis confirms that AR binds to a consensus androgen response element in these core autophagy genes, which are located in the promoter regions of *ATG4B, ATG5* and *LC3B*, and the intron regions of *ATG3, ATG5, ATG7*, and *SQSTM1* [79]. Understanding the part played by AR signaling in phagophore expansion via these autophagy-related genes will help elucidate the roles and mechanisms of autophagy in the development and maintenance of many androgen-responsive tissues, which is an interesting area for future investigation of the connection between autophagy and disease. We previously determined the mechanism of RAB37-mediated autophagosome formation by modulating assembly of the ATG12–ATG5-ATG16L1 complex [59,60]. RAB37, as a novel GTPase, regulates autophagosome formation through a switch between two distinct conformations: the GTP-bound "on" and the GDP-bound "off" forms. RAB37-GTP interacts directly with ATG5 and promotes the interaction of ATG12–ATG5 with ATG16L1. The RAB37-ATG12–ATG5-ATG16L1 complex recruits and lipidates LC3B-I to form active LC3B-II, which accelerates autophagosome formation. The regulation of *ATG3, ATG4, ATG5, ATG7*, and *LC3B* by AR, together with RAB37 GTP-GDP cycling, further facilitates autophagosome formation.

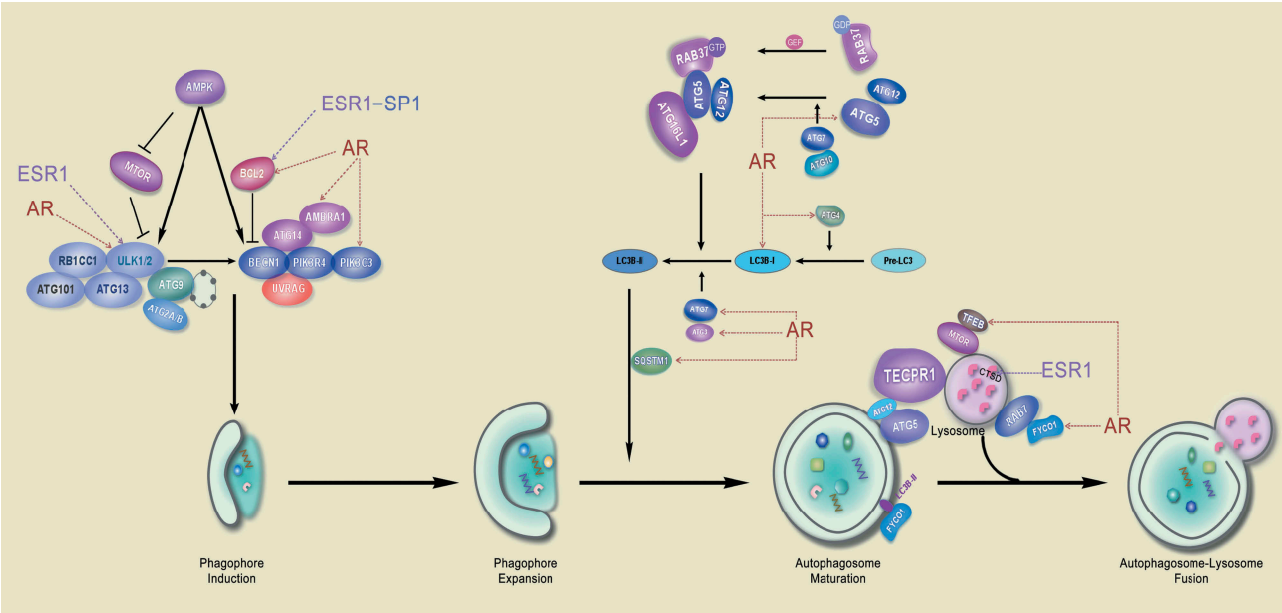


Figure 3. Schematic diagram of the autophagy pathway from phagophore induction, expansion, and maturation, highlighting the transcriptional regulation of autophagy genes by sex hormone receptors AR and ESR1. The regulatory relationship of autophagy genes by AR and ESR1 are indicated by dotted arrows, AR in red and ESR1 in purple.

Thus, AR plays important roles in autophagy via the regulation of these core autophagy genes at the transcriptional level.

In autophagosome maturation, FYCO1 promotes the microtubule plus end-directed transport of autophagic vesicles [80], and AR can bind to three intron regions of *FYCO1* [79] (Figure 4). Mutations of FYCO1 lead to congenital cataracts [81] and autophagy is involved in photoreceptor degeneration and damage [82,83], suggesting a possible role of AR regulation of *FYCO1* in eye development and vision maintenance. In lysosome biogenesis, TFEB plays an important role in linking autophagy to lysosomal biogenesis [84]. AR can upregulate *TFEB* transcription through binding to two androgen response sites of *TFEB*: one site is located in a 5′ region 12 kb upstream from the transcription start site, and the other site is located within an intron. Thus, *TFEB* may at least in part be directly regulated by AR, suggesting a role for this regulation in prostate cancer progression [78]. CTSD is a lysosomal proteinase induced by estrogens, which has been used to improve neuropathology in neuronal ceroid lipofuscinosis [85], and restore autophagic flux and lysosome homeostasis in saposin C-deficient fibroblasts [86]. In the promoter of the human *CTSD* gene, two estrogen-responsive elements have been identified [87], suggesting a role of ESR1 in transcription regulation of *CTSD*.

There are two types of mechanisms for the regulation of autophagy genes by AR and ESRs: regulation by either AR or ESRs, and regulation by both AR and ESRs. For example, *ATG3* is regulated by AR only, whereas *ULK1* can be regulated by both AR and ESR1. The co-regulation by two kinds of sex hormone receptors could occur in the same or different processes. These regulatory mechanisms remain to be further explored. In addition, bioinformatics analysis has suggested numerous autophagy genes are regulated by ESR1 and ESR2 at the level of transcription [76]. There are 12 autophagy genes regulated by ESR2, including *ULK2, ATG7, ATG13, ATG14, ATG16L1, UVRAG*, and *AMBRA1*, and 19 autophagy genes regulated by ESR1, such as *ULK2, ATG5, LC3B, PIK3C3*, and *SQSTM1* (Figure 5). It should be noted, however, that changes in autophagy gene expression regulated by AR and ESR should be tested to confirm sex differences in autophagy and disease, and the changes may not necessarily alter autophagy activity under some conditions of both physiological and pathological processes. Further experimental confirmation of these target genes of sex hormone receptors will help in understanding sex differences in autophagy-mediated diseases.

Future perspectives

Sex differences exist in many types of disease and they are also involved in numerous aspects of disease in mechanism, occurrence, development, therapy, and prognosis. There are many factors that lead to differences in diseases, mainly including genetic and environmental influences. Of them, autophagy is one of the important cellular processes that maintains cellular homeostasis in health and disease, and certain disease-causing sex-related factors are involved in autophagy. Sex differences in autophagy regulation contribute to a higher risk of some types of disease and a greater severity of pathology, but they may also be independent of other sex-related factors in certain situations. It is particularly important to differentiate between these effects in further studies. In addition, whether autophagy plays a causal role in the pathogenesis of certain sex-related diseases remains to be studied in the future.

To date, the underlying molecular and regulatory mechanisms of sex differences in autophagy are poorly understood. Future studies will address signaling pathways, multilayer regulatory mechanisms, including transcription, post-transcription and post-translational modifications, and the

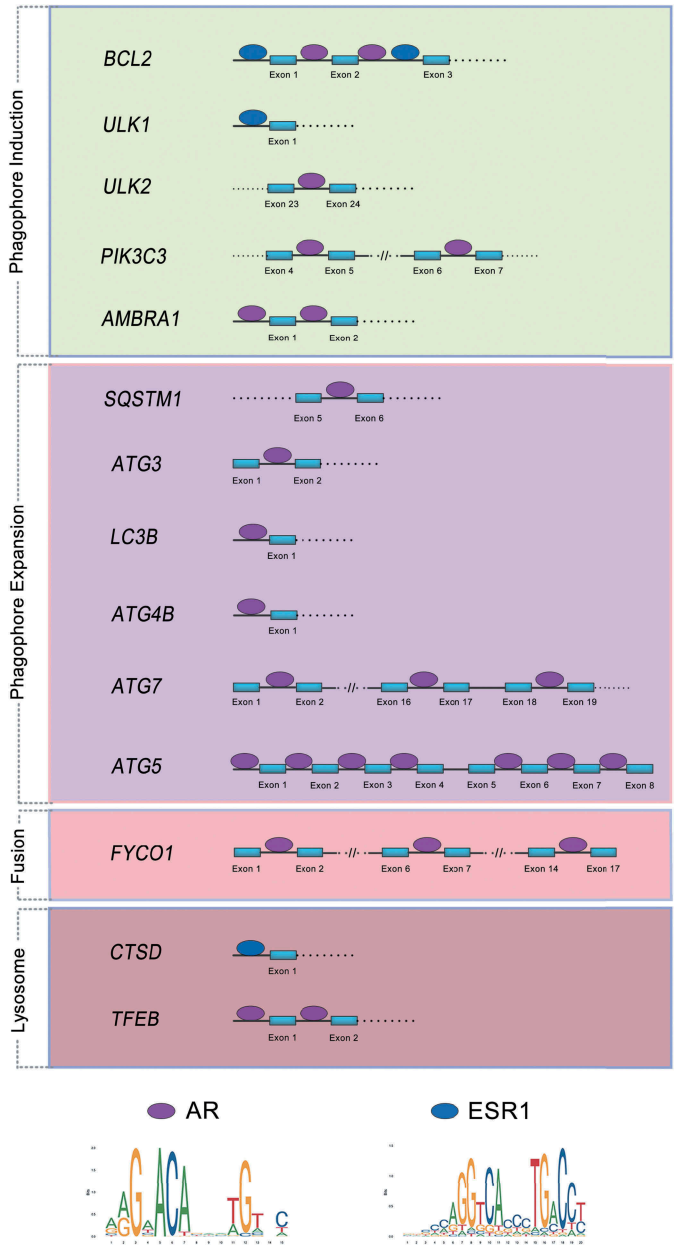


Figure 4. Transcriptional regulation of autophagy genes by sex hormone receptors AR and ESR1 in humans, experimentally confirmed. Autophagy genes for phagophore induction, expansion, lysosome fusion, and lysosome genesis are shown in groups with different colors. The binding sites of the sex hormone receptors AR and ESR1 on autophagy genes are indicated in purple (AR) or blue (ESR1) ovals. The sequence logos of AR and ESR1 for binding sites are shown in the lower panel. The regulatory relationships of autophagy genes by AR and ESR1 were retrieved from references mentioned in the text, and related position information of AR and ESR1 binding was obtained by mapping to the human genome hg38 (http://genome-asia.ucsc.edu/).

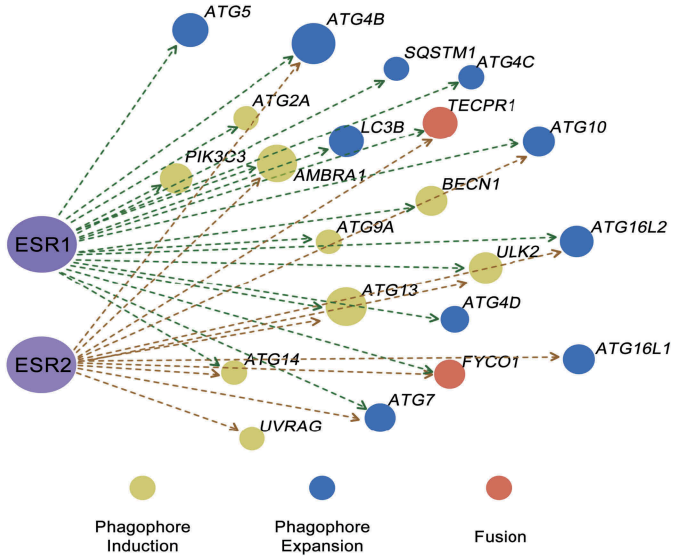


Figure 5. Transcriptional regulation of autophagy genes by sex hormone receptors ESR1 and ESR2 in humans, analyzed with bioinformatics. Autophagy genes for phagophore induction, expansion, and fusion with lysosomes are shown in different colors. The regulations of relationships were predicted through bioinformatics analysis by Türei et al (2015) [76].

interplay of autophagy and the sexes, and elucidate their biological roles in sex differences in diseases. Determining how AR and ESRs precisely regulate autophagy genes in different physiological and pathological processes, examining how and to what extent autophagy contributes to the onset, progression, prevention, therapy, and prognosis of diseases with sex differences, and developing autophagic molecular targets that are different for women and men will be promising directions in this field. For example, manipulating autophagy will be a new therapeutic tool in the treatment of Alzheimer disease. Accordingly, before translation of the knowledge of sex differences in autophagy into pre-clinical practice, relevant standards for diagnosis and therapy should be made for precision medicine.

Considering the important roles of epigenetic regulations in sex differences, the details of epigenetic modifications at the level of genomic DNA (methylation), at post-transcriptional levels (via RNA binding proteins or miRNAs), and at post-translational levels (acetylation, ubiquitination, methylation, phosphorylation, and SUMOylation), and their regulatory effects in autophagy and sex differences, remain to be identified. The m6A modification of mRNA is worth investigating in particular. The m6A modification of mRNA plays an important role in directing mRNAs to distinct fates for development and disease [88]. A recent study determined the roles of m6A modification in autophagy through upregulating ULK1 [89]. On the one hand, the m6A-marked *ULK1* transcripts in the 3′-UTR can be targeted for degradation by YTHDF2, whereas ESR1 can regulate the transcription of *ULK1* [15]. On the other hand, FTO can erase the demethylation of *ULK1* transcripts and upregulate the abundance of the ULK1 protein, thus promoting the initiation of autophagy. As ESR1 can regulate transcription of *ULK1*, m6A modifications are probably involved in a sex-difference regulation, which remains a question for further studies. Given that m6A modifications function during developmental transitions by targeting m6A-marked transcripts for degradation [90], identifying crosstalk between m6A modulators and autophagy will reveal an interesting landscape of autophagy regulation in sex differences in disease, and illuminate the molecular functions and biological importance of m6A modification in disease.

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Disclosure statement

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